Bacterial Genetics
GMS6038
Final Exam
Fall 2009

Make up a code name for yourself and put it here. ___________________________

Write down the code with your name on a sheet for the class so I can grade the exam in a blinded manner.

You have 2 hours to complete the exam.

Please note - this is a closed book, closed note exam. All backpacks and notebooks must be against the wall, not at your table.

You may use the rest room one person at a time.

Any cheating will result in a 0 for the exam and failure of the course.

Shown on the next page is pGULIG-11. Note the following genetic elements:

At the top of the map is the origin of replication of the F plasmid. Since I used both *oriS* and *oriV* in class, I have provided both names here, but they are considered to be synonymous.

Moving clockwise:

From 1:00-2:00 is the *lacI* gene expressed by its own promoter.

From 2:00-4:00 the *rom/rop* (synonymous names) gene is expressed from the wild-type pBAD promoter which is in its natural configuration relative to the *araC* gene, also expressed by its own natural promoter.

At 5:00 is the *oriT* locus of the F plasmid.

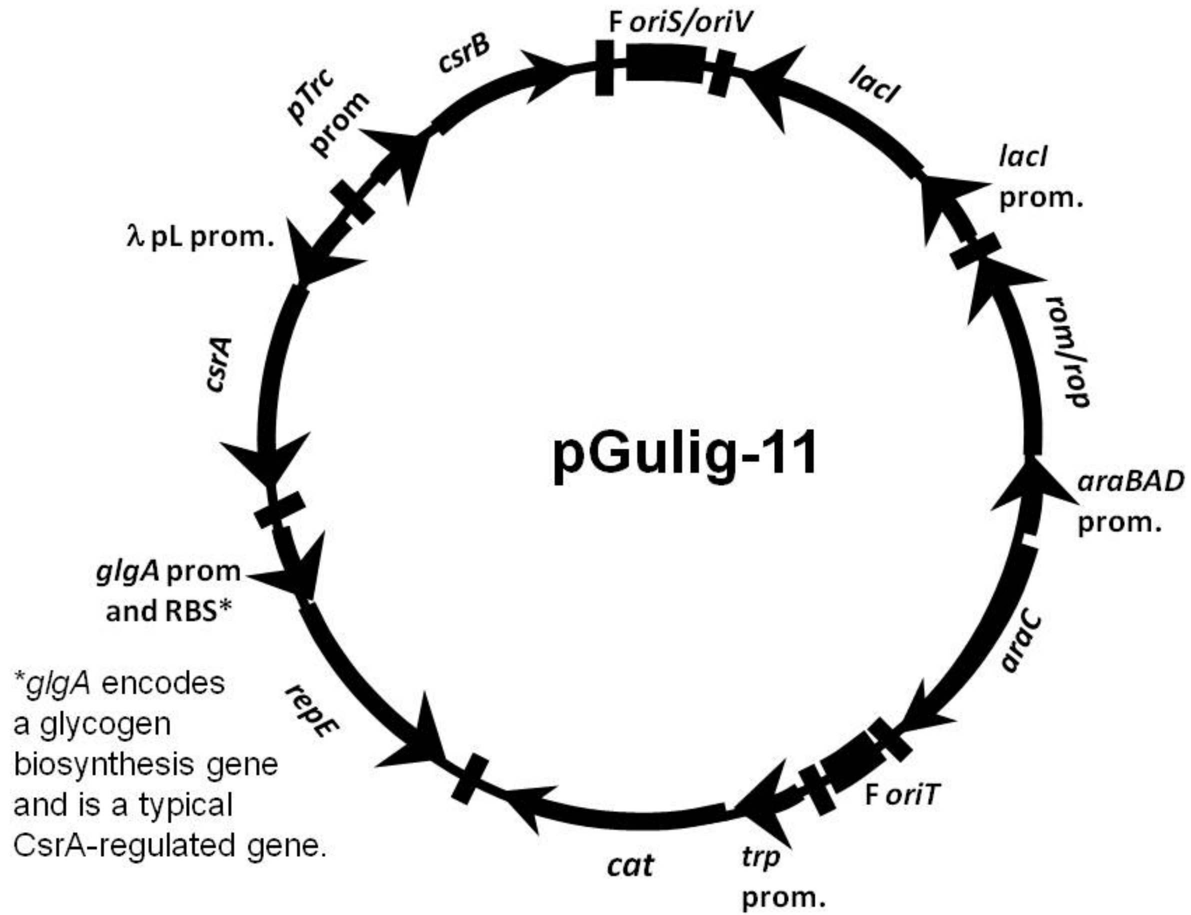
At the bottom is the *trp* (*trpL*, not *trpR*) promoter driving the expression of the *cat* gene.

At 7:00-8:00 is the *repE* gene expressed from the *glgA* gene promoter, including the *glgA* ribosome binding site (RBS). We did not mention the *glgA* gene by name in class. However, all you need to know about *glgA* is that it is a typical CsrA-regulated gene, meaning that it follows the most common relationship between the CsrA protein and a CsrA-regulated gene.

At 9:00 is the *csrA* gene expressed from phage λ pL promoter.

Finally from 10:00-11:00 is the *csrB* gene driven by the pTrc promoter.

There are no other promoters on this plasmid other than those indicated on the map. Assume that all genes have appropriate translation initiation and termination sequences. The short bars crossing the circle represent typical factor-independent terminators that will prevent transcriptional read through between these different elements. Every gene that is present on pGulig-11 has been deleted from the *E. coli* chromosome in the strain that houses pGulig-11. The host *E. coli* strain also **lacks the F plasmid and λ phage**.


pGulig-11
F oriS/oriV
csrB
pTrc prom
λ pL prom.
csrA
glgA prom and RBS*
repE
cat
trp prom.
F oriT
araC
araBAD prom.
rom/rop
lacI prom.
lacI
*glgA encodes a glycogen biosynthesis gene and is a typical CsrA-regulated gene.

NOTE: These questions involve 2 or 3 sentence answers - not paragraphs. The number of points is the maximum number of facts being looked for, and in some cases one fact is worth 2 points. Keep to what is being asked. Adding extra material hoping to include the right answer somewhere in the middle could count against you if your answer reveals a lack of understanding or includes incorrect information, even if unrelated to the original question.

Write your answers legibly on lined paper with your code on each page.

Questions 1 through 6 are based on pGulig-11. Here is a clue to help work these questions. Start at the genetic element that is being asked about and then work your way through the rest of the plasmid looking for genes that are related to those. Don't try to take in the whole plasmid at once. If you believe that a gene/locus is involved with an answer, but you are not sure of its regulation, be sure to mention this to get partial credit.

1. (5 points) If you wanted to express the *rom/rop* gene, what growth conditions would you use? Explain. What would be the effect of expression *rom/rop* relative to replication of pGulig-11? Explain.

arabinose with no glucose. It is expressed by the *ara* system that has arabinose as the inducer. Since it is under catabolite repression, you cannot have glucose present. Rom/Rop repressed ColE1 replication by stabilizing the RNA-I and RNA-II hybrid. However, the ColE1 origin is not present on pGulig-11, so Rom/Rop has no effect.

2. a. (2 points) What conditions are necessary for expression of *csrA*? Explain.

None. The λ pL promoter is constitutive. There is no CI in the cell to repress it.

b. (3 points) What conditions are necessary for expression of *csrB*? Explain.

Add IPTG or lactose – glucose is irrelevant. The Trc promoter is regulated by LacI encoded on the plasmid and expressed by its own promoter. So you must add an inducer to get expression. The Trc promoter is a hybrid that is not under catabolite repression, so glucose is irrelevant.

c. (4 points) How does CsrA affect the expression of the *repE* gene (not just increase or decrease, but what does CsrA do a the molecular level)?

It will cause the mRNA to become less stable or it will directly inhibit translation. It does this by binding to GGA sites in the RBS and elsewhere on the mRNA. The levels of transcription are not affected, but levels of protein decrease.

3. (2 points) What are the growth conditions that are necessary for pGulig-11 to be replicated in the host *E. coli* strain described above? Explain. You do not have to repeat any detail already provided in answers above – just mention the relationships between the conditions and the relevant genetic loci.

Add IPTG. This will enable csrB RNA to be expressed. csrB RNA will bind up the CsrA protein which will not be available to inhibit repE mRNA stability or inhibit RepE translation. RepE will then be able to act at the only origin of replication on the plasmid, F oriS/oriV, to stimulate replication.

4. (3 points) Is pGulig-11 conjugative, mobilizable, or neither in this *E. coli* host? Explain.

Because it has an oriT, it could be mobilizable – IF the F plasmid tra system was present. But this *E. coli* does not have the F plasmid, so pGulig-11 is stuck.

5. (5 points) What antibiotic resistance does this plasmid confer? What growth conditions are essential for expression of the antibiotic resistance? Explain. How does this resistance mechanism work? How does the antibiotic work (i.e., what is its target?).

Chloramphenicol resistance from the *cat* gene. Because it is expressed by the trp promoter, you cannot have tryptophan in the medium. Trp would act as a co-repressor with the TrpR protein (from the chromosome) to cause repression of *cat*. Cat is chloramphenicol acetyl transferase – it inactivates chloramphenicol by acetylating it. Chloramphenicol inhibits protein synthesis.

6. a. (3 points) Could pGulig-11 be used as a suicide plasmid? Your answer should make it clear that you know what a suicide plasmid is. Why or why not?

Yes, in theory it could be a suicide plasmid. In the absence of IPTG, it might not have enough RepE to replicate. A suicide plasmid is one that can be grown under conditions that prevent its replication, forcing the integration into the host genome to maintain the plasmid.

b. (3 points) What would you add to pGulig-11 to either make it a suicide plasmid or make it a better suicide plasmid, depending on your answer above. How would your addition improve pGulig-11 as a suicide plasmid?

Good suicide plasmids have a counterselectable marker so that you select for their absence when the second crossover occurs for allelic exchange. SacB is a good counterselectable marker.

The rest of the questions are independent of pGULIG-11.

7. (3 points) If a *Staphylococcus aureus* strain that was previously sensitive to methicillin became resistant to methicillin (i.e., became a MRSA – methicillin resistant *S. aureus*) in a patient (not just by itself in the lab), which of the following most likely was responsible for this change: spontaneous point mutation, transduction, or acquisition of a *bla* gene on a plasmid? Explain.

Transduction. MRSA is caused by a block of resistant penicillin-binding proteins (PBPs) having been acquired by SA long ago. They cannot be produced by a single point mutation, and they are not known to exist on plasmids. Besides, if a plasmid with these MRSA genes was acquired, the wild-type PBPs would still be inhibited and cause trouble. So the strain most likely acquired the MRSA PBPs by horizontal gene transfer, and transduction is the most likely method.

8. (5 points) For which of the 6 secretion systems in gram-negative bacteria would the secreted protein (target protein) lack a typical Sec signal (leader) sequence? Explain.

Types 1, 3, and 4 (maybe 6 – we don't know enough). These systems secrete the target proteins directly from the cytosol into a host cell, so the target proteins are not exported by the Sec pathway.

9. (3 points) If someone did an experiment that mapped transcription initiation sites, and they told you that the initiation site was 3 nucleotides upstream of the ATG start codon of a particular gene, would be surprised by this result? Explain.

You should be. Most bacterial genes have a ribosome binding site that is 5-10 nt upstream of the ATG codon. This transcript could not encode a RBS. However, in such a case, the gene could be translated by the ATG being so close to the 5' end of the mRNA.

10. (3 points) Why is it important for the third amino acid in the peptidoglycan building block to have an amino group-containing side chain?

It forms the cross-linking transpeptide bond with the fourth amino acid – D-Ala.

11. (4 points) What is the difference between smooth and rough lipopolysaccharide? Which type of change would be easiest to accomplish by a single point mutation – smooth to rough or rough to smooth? Explain.

Smooth LPS has O antigen, Rough LPS ends at the core. A point mutation cannot enable the extra sugars and transferases to be made to produce O antigen. However, a point mutation in any of several genes could inhibit O antigen production of addition to the core.

12. (3 points) If you want *E. coli* to produce your favorite protein from a cloned gene, irrespective of the regulation of the promoter driving the recombinant gene's expression, what growth conditions would you use to get a maximal yield? Explain.

Aerated in log phase are the main things for full credit. 37C and rich broth could also be mentioned.

13. (6 points) List 2 antibiotics that have different spectra (cell types that are affected). Explain the reason for the limited spectrum.

Many answers here: bacitracin and vancomycin are G+ because they are too large to get through G- porins; polymyxin is G- because it binds to LPS

14. (5 points) You have cloned your favorite gene into a pET plasmid that uses a phage T7 promoter to express your gene. How does using this type of plasmid affect the host *E. coli* strain that you should use? Explain. How does using this plasmid affect your use of the cloned gene, i.e., what is this type of expression most often used for? Why?

The *E. coli* strain must encode the T7 RNA polymerase. Because this system causes such high levels of expression, it is used for expression of very high levels of protein (purification for immunization, enzyme activity, etc.). The *E. coli* will be too sick to do things like infect animals.

15. (4 points) In general terms, what is phage display? Why is it useful?

Cloning genes into phages (or phagemids) so that the proteins are displayed on the surface of the phage particle. You can then select from a library of recombinant phages those phages that exhibit the binding activity you want. It is useful for many reasons, but the most important one is that when you isolate the phage with the desired binding protein, you have the gene encoding that protein inside of the phage particle for your use and analysis.

16. (6 points) What be the effects of mutating each of the following λ phage genes be on λ infection of an *E. coli* cell? Explain.

a. CI – no lysogeny since CI represses the pL and pR promoters that are essential for the lytic phase

b. CII – no lysogeny since CII induces the CI gene

c. Cro – no lytic phase since Cro represses CI, and without this repression CI will repress pL and pR

17. (3 points) Your strain of *E. coli* has a doubling time of 30 minutes. If you start with a cell density of 10^3 CFU/mL and stationary phase is at 10^9 CFU/mL, how many hours will it take for the culture to reach stationary phase, assuming that the bacteria start in exponential phase, with no lag phase? Your answer should be within 1 hour and be rounded to the hour. You do not need a calculator to do this, even if you use the mathematical formula taught in class. If you use the "rule of thumb" estimate regarding estimating numbers of generations, your answer will be correct.

9 to 10 hours. There is a 10^6-fold increase in cells. For every 10-fold increase, it takes about 3 generations (rule of thumb). 6x3=18 generations. 18 g ÷ 0.5h/g = 9 h. If you used the g=log(10^9/10^3) ÷ 0.3 you get log(10^6)/0.3 = 6/0.3 = 20 generations. 20 g ÷ 0.5h/g = 10 h.

18. (4 points) Explain how DnaK is involved in its own expression.

DnaK is expressed using the RpoH heat shock sigma factor. DnaK inhibits RpoH by causing its degradation under normal circumstances, hence it inhibits its own expression. Under heat shock, DnaK is tied up with other proteins, enabling RpoH to do its job, and part of that job is transcribing the dnaK gene.

19. (2 points) Why does DNA polymerase I have 5'-3' exonuclease activity, but DNA polymerase III lacks this activity?

Pol I is involved with completing Okazaki fragments which have numerous RNA primers. Pol I must remove the RNA primers with the 5' to 3' exo activity. Pol III does not do this.

20. (4 points) What two ways does the replication rate of bacteria affect the composition/size of the cell? Explain.

Multiple copies of the chromosome make the cells visibly larger. Higher numbers of ribosomes are required to ensure protein synthesis, so by EM you could see more ribosomes.

21. (4 points) Are the F plasmid and ColE1 plasmid compatible or incompatible? Explain?

Compatible. Their replication systems are not related in any way, so they will not interfere with each other's regulation of initiation of replication.

22. (4 points) SRP and SecB are both involved with export of proteins. What is different between the proteins that interact with SRP and SecB in terms of their:
a) signal sequence and b) ultimate location in the *E. coli* cell.

a) The SRP signal sequence is larger since it forms the first transmembrane domain of SRP-associated proteins, which b) usually end up as integral inner (cell) membrane proteins. SecB-associated proteins end up in the periplasm.

23. (6 points) Briefly explain how a factor-independent (Rho-independent) terminator works in *E. coli*. You should include in your answer the essential elements of the terminator sequence.

GC-rich stem-loop followed by poly-U (in the mRNA). The stem-loop causes the weak U-A RNA-DNA hybrid to destabilize which disrupts the transcription bubble.

24. (4 points) How would each of the following mutations affect the sensitivity of the maltose operon to the presence of glucose in the medium? Explain.

a. *cya* gene
b. *crp* (*cap*) gene

Both mutations would not let the maltose genes be expressed, even if glucose was not in the medium. There would be no cAMP because of the cya deletion, and there would be no Crp/CAP protein for the cAMP to bind to, anyway. Therefore, the maltose genes would never be fully expressed.

Extra credit – 5 points – Briefly explain how a marker plasmid works to enable the determination if differential yields of two bacterial strains from an animal infection are due to differential growth of the bacteria or differential killing by the host during infection. You don't have to explain how to make a suicide plasmid, just how it would be used.

The marker plasmid is a conditional suicide plasmid. It can be enabled to replicate in vitro under specific conditions, but those conditions are not met in the animal. The plasmid has a selectable marker such as an antibiotic resistance gene. In the animal, with each bacterial division, since the plasmid is not replicating, the plasmid gets diluted in the total bacterial population with exponential kinetics. When you sample the bacteria from the animal, you plate selectively under plasmid-inducing conditions and nonselectively. The ratio of the plasmid-containing to plasmid-cured cells indicates the number of generations and hence growth rate. Conversely, by enumerating the total number of plasmid-containing bacteria recovered from the animal, you can get an idea of how much killing has occurred, since once a plasmid-containing cell has been killed, the plasmid cannot be recovered. The total number of plasmids recovered from the animal indicated killing.